Wong EKS, Kavanagh D. Anticomplement C5 therapy with eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome. *Translational Research* 2015, 165(2), 306-320.

Anti-complement C5 therapy with eculizumab for the treatment of Paroxysmal Nocturnal Haemoglobinuria and Atypical Haemolytic Uraemic Syndrome

E.K.S. Wong[a] and D. Kavanagh[a]

[a]Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne

Correspondence: Dr Edwin Wong, Institute of Genetic Medicine, International Centre for Life, Central Parkway, Newcastle upon Tyne, NE1 3BZ. Telephone: +44 (0) 191 241 8634; Fax: +44 (0) 191 241 8666; Email: edwin.wong@newcastle.ac.uk

Abstract

The complement inhibitor eculizumab is a humanised monoclonal antibody against C5. It was developed to specifically target cleavage of C5 thus preventing release of C5a and activation of the terminal pathway. Paroxysmal nocturnal haemoglobinuria (PNH) and atypical haemolytic uraemic syndrome (aHUS) are two diseases with distinctly different underlying molecular mechanisms. In PNH, progeny of haematopoietic stem cells that harbour somatic mutations lead to a population of peripheral blood cells that are deficient in complement regulators resulting in haemolysis and thrombosis. In aHUS, germline mutations in complement proteins or their regulators fail to protect the glomerular endothelium from complement activation resulting in thrombotic microangiopathy and renal failure. Critical to the development of either disease is activation of the terminal complement pathway. Understanding this step has led to the study of eculizumab as a treatment for these diseases. In clinical trials, eculizumab is proven to be effective and safe in PNH and aHUS.

Abbreviations

AE	Adverse events
aHUS	Atypical haemolytic uraemic syndrome
AP	Alternative pathway
C3bB	Proconvertase
C3bBb	C3 convertase
CKD	Chronic kidney disease
CP	Classical pathway
CR1	Complement receptor 1
DAF, CD55	Decay acceleration factor

DGKε	diacylglycerol kinase ε
FB	Complement factor B
FD	Complement factor D
FH	Complement factor H
FI	Complement factor I
GAG	Glycosaminoglycans
GPI	Glycosyl phosphatidylinositol
GPI-AP	Glycosyl phosphatidylinositol anchoring protein
IgG	Immunoglobulin class G
LDH	Lactate dehydrogenase
LP	Lectin pathway
MAC, C5b-9	Membrane attack complex
MCP, CD46	Membrane cofactor protein
NO	Nitric oxide
PEX	Plasma exchange
PIGA	Phosphatidylinositol glycan class A
PNH	Paroxysmal nocturnal haemoglobinuria
SNP	Single nucleotide polymorphism
Stx	Shiga-toxin
TMA	Thrombotic microangiopathy
TTP	Thrombotic thrombocytopaenia

Introduction

Eculizumab remains the first and only inhibitor of the complement system used in clinical practice. Specifically, it targets the terminal complement pathway and leaves the proximal complement pathways intact. It was approved by the United States Food and Drug Administration and the European Medicines Agency in 2007 for the treatment of paroxysmal nocturnal haemoglobinuria (PNH) and in 2011 for the treatment of atypical haemolytic uraemic syndrome (aHUS). This review discusses the current use and potential limitations of eculizumab as a therapeutic agent.

The complement system

The complement system comprises a network of proteins that work in concert as part of the innate immune system and has been extensively reviewed (1). Their role in orchestrating the immune response extends beyond the concept of 'first line of defence'. It bridges innate and adaptive immunity (2) and plays a key role in removal of immune complexes and injured cells and tissues (3).

Briefly, and summarised in figure 1, complement can be activated via the classical pathway (CP) through antibody-antigen interactions and lectin pathway (LP) through pattern recognition molecules expressed on the sugar coating of bacteria. These lead to the formation of the C3 convertase of the classical pathway leading to cleavage of C3 into C3a and C3b. Complement also activates via the alternative pathway (AP) through hydrolysis of C3 into $C3_{H20}$ by a process known as 'tickover'. This binds complement factor B (FB) to form $C3_{H2O}B$ which is then converted to $C3_{H2O}Bb$ by the action of complement factor D (FD). This initial convertase can also cleave C3 into C3a and C3b.

C3b from any activation pathway then enters the amplification loop of the AP by binding FB to form a proconvertase (C3bB). In the presence of FD, C3bB is cleaved to form the C3 convertase of the alternative pathway (C3bBb) which again cleaves C3. C3a is released in its effector role as an anaphylatoxin. C3b binds FB to feed back into a positive amplification loop resulting in the massive generation of C3b for opsonisation.

As the density of C3b on cell surfaces increases, convertases with an extra C3b subunit form and change specificity from C3 to C5 allowing cleavage of C5. In this terminal pathway, cleavage of C5 leads to the production of C5a and C5b. C5a instigates inflammation whilst C5b binds C6, C7, C8 to form C5b-8 as part of the lytic pathway before a final protein C9 completes the formation of the membrane attack complex (MAC, C5b-9). In total 80-90% of complement activation results from the amplification loop of AP regardless of the initial mechanism of complement activation (4).

Given that the AP is spontaneously active, there are complement regulatory proteins to prevent bystander complement activation and amplification upon host cells and surfaces. These are found in the circulation and on cell membranes. They have several functions and are described below. Acceleration of natural decay of C3bBb (decay acceleration) by complement factor H (FH), decay acceleration factor (DAF, CD55) and complement receptor 1 (CR1); co-factor activity to allow the complement factor I (FI) mediated cleavage of C3b into iC3b by FH, CR1 and membrane co-factor protein (MCP, CD46) and subsequent degradation of iC3b into C3dg by CR1. In the terminal lytic pathway, the complement regulator CD59, can block C9 binding to C5b-8 thus preventing the formation of MAC.

Loss of complement regulation resulting in the inability of complement to protect host tissue from complement activation has underpinned the pathogenic mechanism in several diseases including PNH (5) and aHUS (6) (Table 1). These abnormalities of complement regulation include somatic mutations in haematopoietic stem cells in PNH and germline mutations in complement genes in aHUS and are described in more detail later. Through an understanding of these pathogenic mechanisms, including the importance of the terminal pathway common to both PNH and aHUS, there have been benefits from the use of the complement inhibitor eculizumab.

Eculizumab

Development of an anti-C5 antibody began by screening murine monoclonal immunoglobulin (IgG) that bound to human C5 for the capability to block C5a generation and complement mediated cell lysis via MAC (7). To reduce immunogenicity of murine IgG, the antibody was humanised by cloning the variable regions into human germline heavy-chain and light chain (8). Human IgG activates complement and has a pro-inflammatory effect upon binding to its target – to minimize this, the hybrid region utilises the desirable properties both of IgG2, which fails to bind Fc receptors (9), and IgG4, which does not activate complement to reduce the pro-inflammatory potential of the antibody (10). This final recombinant antibody to C5 is now known as eculizumab and retains the ability to block C5a generation and cell lysis (8).

Preclinical and early clinical development

Eculizumab is species specific and not effective in murine models. Therefore in early animal testing, a surrogate mouse anti-C5 antibody was used to study the effects of MAC inhibition in murine models of both collagen-induced arthritis (11) and lupus-like autoimmune disease in NZB/WF1 mice (12). The results provided safety and efficacy data that supported the feasibility of using an antibody therapeutically for sustained blockade of C5.

Initial clinical development

Initial phase 1 studies in both systemic lupus erythematosis (13) and rheumatoid arthritis (14, 15) showed that a dose of 8mg/kg blocked complement for 7-14 days. In the small numbers of patients studied, there was a trend towards clinical improvement. There was no dose dependent trend in adverse effects. Subsequently, complete blockade of the terminal pathway of complement was demonstrated in vivo with serum concentrations above 35 µg/mL (16).

Eculizumab and risk of meningococcal infection

Patients with deficiency of terminal pathway components including C5 strongly associate with infections with encapsulated organisms including *Neisseria meningitides* (17). Therefore all patients receiving eculizumab should receive vaccination against all known strains. If eculizumab needs to be commenced within 14 days of vaccination, additional prophylactic antibiotics should be given. Until recently, vaccination was not available for *Neisseria meningitides* serotype B, the most common strain in Europe. Many centres currently recommend prophylactic antibiotics for the duration of eculizumab therapy.

Paroxysmal Nocturnal Haemoglobinuria

Paroxysmal nocturnal haemoglobinuria is a rare haemolytic anaemia first described in 1882 (18). Due to intravascular haemolysis, the appearance of haemoglobin in the urine gives rise to its current name, although this observation is a presenting feature in only 26% of patients (19, 20). The first diagnostic test for PNH was demonstrated in the original 'acidfied serum assay' or Ham test (21) and the underlying basis that complement is important in haemolysis was shown in 1954 (22). The additional disease associations of thrombosis, muscle dystonias, chronic kidney disease and bone marrow failure pre-date the discovery of the underlying mechanisms demonstrating that PNH is not merely a disease of erythrocytes (5).

Pathogenesis

PNH results from the non-malignant clonal expansion of haematopoietic stem cells that contain a somatic mutation in the gene *phosphatidylinositol glycan class A* (*PIGA*) found on the X-chromosome (23). *PIGA* encodes for a glycosyl transferase that is required in the biosynthetic pathway for the synthesis of glycosyl phosphatidylinositol (GPI) (24). Progeny of haemopoietic stem cells containing a *PIGA* mutation (erythroid, lymphoid and myeloid) harbour the same mutation leading to a population of peripheral blood cells that are deficient in GPI-anchored proteins (GPI-AP).

All cells have the potential to develop a somatic mutation in *PIGA* leading to GPI-AP deficient cells. In fact GPI-AP deficient cells have been identified in healthy controls but these are due to somatic mutations in a fully differentiated cell line and cannot self-renew (25). Somatic mutations in *PIGA* also occur in patients with bone

marrow failure (26). The main question is why the *PIGA* mutant clone should expand preferentially compared to normal haemopoietic stem cells. A two-step hypothesis has been suggested. The *PIGA* mutation itself is an essential first step in disease. However, secondary mutation (27) or resistance to apoptosis (28) might give these cells a survival advantage and explain in some patients why clonal selection should occur. In the case of the latter, GPI-AP deficiency itself may be important in helping the cell evade pro-apotoptic signals in certain circumstances whereas normal cells may not, allowing the mutant PIGA clone to expand (29, 30).

In addition, patients have phenotypic mosaicism of PNH erythrocytes. Cells from an individual clone may be completely deficient in GPI-AP (type III PNH erythrocytes) or partially deficient (type II PNH erythrocytes) and exhibit approximately 10% of GPI-AP. Cells that have normal expression of GPI-AP are termed type I erythrocytes (20). Individuals with PNH will have erythrocytes of a combination of the three subtypes.

Underlying haemolysis and the development of anaemia in PNH is the absence of two GPI-anchored complement regulatory proteins CD55 and CD59 from erythrocytes. Deficiency of these complement regulators is critical to PNH erythrocytes being susceptible to complement-mediated attack. In general, type III cells will undergo spontaneous lysis. Type II cells have sufficient expression of CD55 and in particular CD59 to avoid spontaneous lysis (31) though if type II cells predominate in the circulation, brisk haemolysis may occur in the setting of a trigger that enhances complement activation such as trauma, infection, pregnancy (20). Haemolysis in turn leads to free haemoglobin and nitric oxide depletion that can have consequences that extend beyond anaemia. Whilst it isn't completely clear why individuals should have clones exhibiting different degrees of GPI-AP deficiency, it is

clear that those patients who develop the largest population of type III erythrocytes will have more clinically significant disease (23).

The proposed mechanisms of thrombosis in PNH are multiple and a complete description is beyond the scope of this review. Nonetheless the absence of GPI-AP on the platelet surface is likely to play a strong role in contributing to thrombotic risk as reviewed by Hill et al (32).

Even though there is MAC formation upon the platelet surface due to CD59 deficiency platelet survival in PNH appears to be normal (33). Instead, both MAC formation (34) and some downstream effects of haemolysis (35)(circulating free haemoglobin and nitric oxide (NO) depletion) can result in platelet activation that is associated with increased thrombotic risk (36). The absence of other GPI-AP (including urokinase-type plasminogen receptor (37), and tissue factor pathway inhibitor (38)) are also thought to play a potential role in the mechanisms contributing to thrombotic risk.

Chronic kidney disease (CKD) is a consistent sequelae of PNH (39). Free haemoglobin from haemolysis has been shown to increase renal accumulation of haemosiderin, leading to tubulointerstitial inflammation and kidney damage (40). Free haemoglobin also results in NO depletion that could deleteriously affect vascular tone, particularly in the afferent arterioles of the glomerulus with a negative effect upon glomerular filtration rate and renal blood flow (41). Other consequences that are thought occur due to NO depletion are pulmonary hypertension, gastrointestinal dystonia and erectile dysfunction (35).

Diagnosis

In patients with PNH, laboratory tests reveal Coombs' negative intravascular haemolysis and raised lactate dehydrogenase (LDH). The minimal essential criteria for diagnosing PNH (20) are using flow cytometry to determine the prevalence GPI-AP deficient blood cells (42) and assessment of bone marrow function. GPI-AP deficiency is measured on erythrocytes and polymorphonuclear leukocytes. Due to haemolysis, measurement of GPI-deficiency on erythrocytes alone would underestimate the size of the clone. The anaemia in PNH patients is contributed to by haemolysis and the extent of any associated bone marrow failure. (20).

Symptoms and prognosis

The symptoms of PNH are predominantly constitutional (fatigue, lethargy, malaise). Nocturnal haemoglobinuria is present in only 25% of patients. Additional symptoms may include dysphagia, odynophagia, abdominal pain and male impotence, typically at times of dramatic haemolysis.

PNH is a highly co-morbid condition due to the complications of anaemia (43), thrombosis (32) and chronic kidney disease (39). Thrombotic events are common in PNH. A thrombotic event is usually reported in 29% to 44% of PNH patients are the major cause of mortality accounting for 40% to 67% of deaths for which the cause is known (32). Thrombus develops at unusual sites such as visceral (hepatic vein, mesenteric, portal, splenic, inferior vena cava) or cerebral veins; arterial thrombosis is less common. In 19% of cases, visceral thrombi precede the diagnosis of PNH.

There is geographical variation in thrombosis rates though the exact mechanisms are unknown. African American or Latin American PNH patients had an increased risk of thrombosis compared with other PNH patients (44) .In a

comparision between white American and Japanese PNH patients, a lower risk was reported among Japanese patients (45).

Survival in the setting of thrombotic events is poor and patients with thrombosis at presentation have a 40% survival at 4 years (32), an increased relative risk of death of five - to 15.4 fold. Chronic kidney disease was identified in 65% of patients in a study cohort with advanced kidney failure contributing to morbidity and mortality of PNH (46).

Treatment of PNH

Prior to the introduction of eculizumab, the treatment was largely supportive and reserved for symptomatic patients (20). These patients received blood transfusion on a frequent basis. In some patients, corticosteroids ameliorated haemolysis rapidly and their use was considered of potential use during acute exacerbations only rather than in long-term chronic use to keep toxic effects to a minimum (20). In the event of a thrombotic event, standard anticoagulation was used. In cases of refractory anaemia, severe thrombotic events or severe aplastic anaemia, bone marrow transplantation could be considered.

Eculizumab use for haemolytic anaemia in PNH

PNH erythrocytes lack CD59 and are vulnerable to MAC formation and lysis on the cell surface leading to haemolysis. Therefore inhibition of the terminal pathway of complement was a target for therapy and eculizumab was considered as a potential therapeutic. The main aim of the three clinical trials (47-49) published to date of eculizumab in PNH was to determine efficacy of eculizumab in managing the intravascular haemolytic anaemia. Accordingly, patients were entered into the study

if they had >10% GPI-AP deficient cells (type III), ongoing haemolysis and transfusion requirements. Previously published trials and studies looking at the treatment of anaemia have been summarised in table 2. Patients who received eculizumab had ongoing albeit low transfusion requirements and much lower haemolysis rates with LDH on average slightly above the upper limit of normal. This observation is seen in the various single-centre studies too reflecting the off-trial experience and use of eculizumab in the clinician's hands (50-54) including a study of a paediatric cohort (55).

A further observation is an increase in the size of the GPI-AP type III erythrocyte clone due to increased survival. There is a concern that this could lead to dramatic haemolysis in the event that eculizumab was stopped. Brodsky et al (49) identified 19 patients out of 195 who had discontinued eculizumab following the clinical trial setting. Withdrawal from eculizumab did not so far lead to severe haemolysis in these patients.

Factors that predict a favourable response were studied by Dezern et al (54). Patients who had the best response resulting complete remissions (defined as stable and normal haemoglobin without evidence of haemolysis) had a fall in size of type III erythrocytes. Other factors that could lead to a poorer response included concurrent systemic inflammation and more severe underlying bone marrow failure.

Eculizumab and thrombosis in PNH

In the UK study (50), 34 thrombotic events were observed in 21 patients before starting eculizumab. Seventeen events occurred whilst on anticoagulation supporting the theory that anticoagulation alone does not prevent thrombosis. There were just 2 events occurring whilst on eculizumab. The thrombotic event rate before and after

eculizumab was 5.6 vs 0.8 per 100 patient years, a statistically significant reduction. More specifically, out of 7 patients who had a thrombosis prior to commencing eculizumab, none have had further events whilst on eculizumab. Furthermore, in 21 patients who have stopped primary prophylactic anticoagulation since commencing eculizumab, none have developed thrombosis.

In the extension study of the three main trials (56), eculizumab led to an 81.4% decrease in thrombotic events in the extension study of eculizumab, a statistically significant change. In patients who subsequently stopped anticoagulation; none developed a further thrombotic episode. Therefore, although thrombotic events were not an outcome measure in the studies, the data suggests that eculizumab does reduce thrombosis in PNH patients.

Eculizumab and CKD

Data from patients in the TRIUMPH study compared renal function from those treated with eculizumab and those with placebo. Eculizumab significantly improved kidney function over 6 months (46). Improvement in renal function could be shown for all 195 patients recruited into the extension study and the effect was greatest in those patients with Chronic Kidney Disease stage 1 or 2 (milder renal impairment).

Other benefits of Eculizumab in PNH

In a study involving the patients originally enrolled in the TRIUMPH study, it as shown that intravascular haemolysis caused a state of NO catabolism leading to pulmonary hypertension that could be corrected using eculizumab. Dyspnoea was improved independent of anaemia status (57). There have been individual reports where there were improvements in gastrointestinal symptoms (58).

Eculizumab and survival

In the extension study of 195 patients from the original clinical trials, there were 4 deaths. The Kaplan-Meier estimate of survival at 36 months was 97.6% that was sustained out to 66 months. None of the deaths were considered related to eculizumab treatment. (56). This survival rate is similar to the 5-year rate reported in the single-centre retrospective analysis of 79 patients by Kelly et al (50). These survival rates are a marked improvement when compared to historical rates of 65% survival 5 years in patients with ongoing haemolysis (43)

Breakthrough of haemolysis

The standard maintenance dose in PNH is 900mg every 14 days and should maintain a trough drug level of 35µg/ml, sufficient to prevent MAC formation. In 21 out of the 195 patients in the extension study that reported breakthrough haemolysis, shortening the interval between doses was sufficient to maintain inhibition of haemolysis (56). Clearly monitoring for breakthrough of haemolysis is important as part of patient monitoring. In the case of persistent breakthroughs, Hillmen et al (56) recommend testing for human anti-human antibodies or neutralising antibodies. Two of the patients in the long-term extension study were found to have low-positive antibody level – in both cases there was no impact on the function of eculizumab.

Limitations of Eculizumab in PNH

The consistent finding that there was ongoing (albeit minimal) haemolysis has led to further studies. Eculizumab has led to an increase in circulating PNH erythrocytes. Risitano et al (59) and Hill et al (60) studied these PNH erythrocytes in

patients treated with eculizumab and compared to non-treated PNH erythrocytes. PNH erythrocytes treated with eculizumab had evidence of a positive direct antiglobulin test. These cells were then examined on flow cytometry demonstrating the presence of C3b on the cell surface. This finding was unique to Type III PNH erythrocytes and did not occur in type II (or Type I) cells. The likely explanation for this is that Type III PNH erythrocytes are completely deficient in CD55 therefore unable to regulate formation of C3bBb on the cell surface. This allows ongoing deposition of C3b through C3bBb and the alternative pathway. These erythrocytes become heavily coated with C3b that eventually leads to removal via the reticulo-endothelial system leading to extravascular haemolysis. This is likely an exaggeration of an effect that was already occurring in the natural history of disease but masked by rapid intravascular haemolysis.

Rondelli et al (61) then looked at the potential role of C3 and CR1 polymorphisms to explain why some patients were more likely to develop extravascular haemolysis. The hypothesis was that certain polymorphisms including that of the C3 F/S may affect the ability of C3bBb to form on GPI-AP type III cells. They identified the potential role of the High/Low genotype in HindIII of CR1. In eculizumab treated PNH cells, they found that the binding of C3 fragments to erythrocytes was highest for the Low/Low genotype. This suggests that CR1 density on the cell surface has a role in protecting against C3 binding. This was a study limited to erythrocytes from a few patients. This and other mechanisms leading to C3 binding require further study.

Other complement modulator treatments in PNH

Eculizumab blocks the lytic pathway and overcomes the complications of CD59 deficiency. However, the absent CD55 remains a problem in eculizumab treated cells resulting in opsonisation of cells with C3b. This has led to several novel potential therapeutic agents being investigated targeting C3 and the activity of the C3 convertase. In-vitro study of potential therapeutic agents now include monoclonal antibody against C3b (62), a mini-FH comprising the regulatory and recognition domains of FH (63). a novel fusion protein TT30 (64), a peptide inhibitor of C3 (Cp40)(65) and aurin tricarboxylic acid (66).

Eculizumab in clinical use for PNH

Eculizumab is currently recommended in PNH patients with ongoing transfusion requirements, thrombosis related to PNH, haemolysis related complications such as CKD, pulmonary hypertension, and during pregnancy with concurrent haemolytic PNH (67). PNH is a disease of chronic and ongoing haemolysis so treatment is usually recommended lifelong. Patients with asymptomatic PNH clones should be monitored for changes in the size of clone and for the onset of symptoms. In some patients, clone size may spontaneously decline.

Atypical HUS

Atypical haemolytic uraemic syndrome is the prototypal disease of over-activation of the alternative complement pathway (68). Microangiopathic haemolytic anaemia, thrombocytopaenia and acute kidney injury are the hallmarks of haemolytic uraemic syndrome. Renal biopsy reveals the characteristic pathological finding of thrombotic microangiopathy (TMA). Atypical haemolytic uraemic syndrome describes

those cases of HUS that are not caused by Shiga-toxin (Stx) resulting from certain diarrhoeal illnesses such as Escherichia coli 0157:H7 (69).

Pathogenesis

Complement activation due to abnormalities of the complement system are the hallmark of aHUS. Loss of function mutations in complement regulators and gain of function mutations in complement components have been described in aHUS. Additionally, autoantibodies to complement regulatory proteins have been identified. A complete description of all of the genes potentially involved, the numerous mutations and the insight gained into mechanisms of disease have been extensively reviewed (6, 70) and are beyond the scope of this review. However, important points are summarised below and in table 3.

Factor H is the major fluid phase regulator of complement and is characterised by an N-terminal regulatory domain that binds to C3b and carries out its regulatory functions of decay acceleration and co-factor activity. There is also a distinct binding domain at the C-terminus of FH that binds to C3b and glycosaminoglycans (GAG) (71) such as those residing upon the glomerular endothelium. Critically, many of the mutations that have been identified in FH are in this domain suggesting the importance of this C-terminal domain in recruiting FH to the glomerular endothelial surface (72).

Mutations in any of *FH* (72, 73), *FI* (74) and *CD46* (75) can lead to loss of expression (type 1 mutation) or a loss of function (type 2 mutation) as measured in serum levels or functional experiments respectively and therefore resulting in loss of regulation of complement.

C3 and *FB* are part of the activation system of complement. Important mutations in these genes lead to over activity usually by increased formation of C3bBb or resistance to decay acceleration (76, 77).

Autoantibodies against complement regulators have been identified in aHUS. Most commonly they are directed against an epitope mapped to the C-terminus of FH (78). These are usually shown to block the ability of FH to bind to C3b or GAGs and therefore inhibit complement regulation at the glomerular endothelium. Some antibodies bind to other regions or FH and the significance of these in disease are less certain (79). Antibodies have been described against FI in a few cases (80). The functional significance of these has not been confirmed.

Single nucleotide polymorphisms (SNPs) have been described in FH that associate with a 2-4-fold increased risk of aHUS. A haplotype in *FH* (*FH-H3*; *tgtgt*) contains the SNP V62I (81, 82). Functional analysis has shown the V62 risk variant has a small decreased in cofactor activity compared to I62. A haplotype block in *CD46* (*CD46ggaac*) comprises two SNP in the promoter region that associates with a 2 to 3-fold increased risk of aHUS (82). The functional effect of this is less clear.

Penetrance of disease has been reported at around 50% for individuals carrying a single genetic mutation (83) suggesting that additional disease risk modifiers are important. In fact, 3.4% of patients have one or more mutation, with increased penetrance per extra mutation (84). Thus patients have an increased risk of disease with inheritance of mutations and risk haplotypes. Together, these still do not explain why some patients develop disease until later in life. This is best explained by the need for an environmental trigger such as infection (non-Stx), drugs and pregnancy (6).

Diagnosis

Patients with aHUS have low haemoglobin, appearance of schistocytes on blood film, raised LDH, low platelets and raised creatinine. It is important to exclude Stx-HUS by testing for E-coli 0157 on stool culture and PCR. ADAMTS13 levels are also measured to exclude thrombotic thrombocytopaenia (TTP) to allow early appropriate therapy. Genetic and immunological testing for complement abnormalities should be performed. DNA should be tested for mutations in *C3*, *FB*, *FH*, *FI*, *CD46* and copy number variation. Most centres now also suggest testing for mutations in DGKε, encoding a protein not in the complement system. Serum should be sent for C3, C4, FH and FI and complement antibody screening prior to initiation of plasma exchange. FACS analysis of peripheral blood mononuclear cells for CD46 should be performed.

It should be noted that Selier-leclrec et al (85) describe some patients within an aHUS cohort that presented with haematuria and proteinuria without renal failure or ‘uraemia’. The possibility of an underlying complement defect should be considered if there is evidence of a TMA, even without a raised creatinine, potentially in the setting of extra-renal manifestations (see below).

Symptoms and prognosis

Patients with aHUS typically present with symptoms of lethargy, malaise, oedema consistent with the onset of acute kidney injury. They may report prior infective symptoms reflective of a trigger.

There are extra-renal manifestations that have been reported in 10-20% of patients. Most commonly (10%) these are neurological and range from mild irritability to coma (6). Other rare symptoms have been reported in individual case reports and are summarised in (6). The extent to whether these extra-renal manifestations are due to TMA is less clear. In a study of patients at autopsy, patients historically diagnosed with HUS had evidence of fibrin-rich thrombus in brain, heart and pancreas tissue suggesting that these are extra-renal manifestations of HUS. However, these were not distinguished from TTP by ADAMTS13 activity or analysis of the complement system (86).

Historically, aHUS was a progressive disease with poor prognosis with 48% of children and 67% of adults dying or reaching end stage renal failure within 5 years (87). Patients with a mutation in *MCP* generally had a better prognosis, those with mutations in *FH*, *FI C3* and *FB* had similarly poorer prognosis (87). In those patients that receive kidney transplants, disease recurrence rates leading to graft failure are 60-70% (88). Again, patients with mutations in *CD46* have the lowest rates of recurrence; mutations in the other complement genes predict the highest recurrence rates.

Treatment of aHUS

Prior to the introduction of eculizumab the gold standard for therapy was plasma exchange (PEX). Replacement of ineffective or deficient regulatory proteins or the removal of neutralising antibodies and hyperfunctional complement proteins made PEX the logical choice for therapy. Once haemolysis was controlled, PEX

could be withdrawn. Despite this, the prognosis in such patients remained poor and progression to end stage renal failure was common with rates as stated earlier.

Eculizumab use in aHUS

Pickering et al (81) generated a transgenic mouse lacking the C-terminal recognition domains of FH leading to these mice spontaneously developing aHUS. When crossed with a C5 deficient mouse (89), these mice did not develop aHUS suggesting the importance of a role downstream of C3b generation. Therefore, regardless of trigger and underlying genetic mutation, there is evidence that complement activation converges on the terminal pathway suggesting the potential role of eculizumab in aHUS.

The first reports describing the use of eculizumab in aHUS were published in 2009 (90, 91) describing the effect of eculizumab in achieving remission from thrombotic microangiopathy in patients with aHUS in native kidney and also in recurrent disease in a renal transplant. This and other initial reports are summarised in Wong et al (92). Most were successful in achieving remission but of course subject to publication bias.

Following these initial studies were the phase 2 trials of eculizumab (93) summarised in table 4. Two prospective open-label single arms studies were set up and enrolled patients that either 1) had progressive haemolysis despite PEX (C08-002) or 2) had control of haemolysis but remained dependent upon ongoing PEX therapy (C00-003). Seventeen patients were entered into C08-002 and twenty into C08-003. Patients with aHUS affecting native kidney or transplanted kidney were included in both. Subsequently results from a single centre study of off label use of eculizumab in 19 patients (native kidneys) have also been published (94).

The result from these studies show that eculizumab quickly established remission of TMA resulting in normalisation of platelet count, haemoglobin with improvement in renal function (including some who required dialysis). In the two phase 2 trials, this was observed in the first 26 weeks of the initial trial and was sustained during the first extension phase (93). In the off-label study, all patients had disease remission during the follow up period of 4-22 months (94).

This was confirmed in a paediatric study of 15 patients (C09-001) (95) of whom 14 were treated for aHUS in the native kidney. The remaining patient had treatment of recurrent disease in a kidney transplant. Again, 80% achieved a TMA-free status with no patients proceeding to needing dialysis.

Patients who have required dialysis for several months have benefited from eculizumab recovering to independence from dialysis (96). The duration that eculizumab should be continued for once a patient requires dialysis to allow potential recovery is less clear.

Eculizumab as pre-emptive treatment in renal transplantation

Another cohort that appears to benefit from eculizumab is the group of patients who have been classified as high risk of disease recurrence in a renal transplant due to mutations. Rather than wait until recurrence before instigating treatment, patients were given eculizumab at the time of transplantation as pre-emptive therapy. Published reports of this use in 10 patients were reviewed by Wong et al (92). All had mutations that predict a high risk of recurrence and 2 had had previous recurrence in transplant. All patients remained free from disease recurrence at latest follow up of up to 2 years, though one had graft loss due to arterial thrombosis.

Discontinuation of Eculizumab in patients in remission

Within a cohort of French patients that had been successfully treated with eculizumab leading to resolution of TMA and recovery from dialysis requirement, 10 patients had chosen to discontinue eculizumab (97). These patients were monitored for urinary abnormalities using urine dipstick. Amongst these patients, all had *FH* mutations and 8 had previously required dialysis. They had received eculizumab for a median of 5.6 months (range 0.4-14.2). Out of 5 patients that developed haematuria, only 3 had clinically significant disease showing other evidence of haemolysis. These relapses occurred within 6 weeks of discontinuation of eculizumab with associated elevation in creatinine. All 3 patients responded rapidly to restarting eculizumab with resolution of haemolysis and kidney injury. The total (cumulative) time off eculizumab for the entire cohort was 95 months (median, 9.3; range, 0.9-22.7) months. The other 7 patients remain in remission off eculizumab.

Limitations of Eculizumab in aHUS

In addition to abnormalities of the complement pathway, other pathways of disease are being identified in aHUS cohorts. Most notable is the finding of homozygous or compound heterozygote mutations in *diacylglycerol kinase ε* (DGKε) in young affected patients (98, 99). *DGKε* encodes a phosphorylase and is likely to affect the prothombotic pathway. There is no known association with the complement pathway and therefore eculizumab is unlikely to be effective in this cohort. This was reported in the study by Lemaire et al (99). It is plausible that other non-complement pathways exist in aHUS.

For this reason, George and Nester (100) avoid the term aHUS altogether and suggest the term complement-mediated HUS as well as coagulation-mediated HUS.

The limitations that we describe are probably due to the usefulness of the historical term aHUS rather than that of Eculizumab itself. Use of the term complement-mediated HUS may represent a future direction in classification though further discussion of this is beyond the scope of this review.

Clinical use of Eculizumab in aHUS

Eculizumab is recommended for use in patients with aHUS once stool or blood samples have ruled out Stx-HUS and ADAMTS13 levels >10% have been confirmed to exclude TTP.. Plasma exchange may remain the initial treatment until this has been achieved. In paediatric patients where PEX can be difficult to administrate, eculizumab may be used as a first line therapy. Commencement of eculizumab need not wait for confirmation of a genetic or acquired complement abnormality, though the results of these tests may determine the duration of treatment (see below)

Patients that undergo transplantation with high risk mutations (e.g. *FH*, *C3* and *FB*) have unavoidable ischaemia–reperfusion injury induced complement activation making pre-emptive eculizumab the treatment of choice in our opinion.

Family members with a mutation have a low penetrance rate but disease onset may occur when exposed to a triggering stimulus. A strategy of careful monitoring is recommended for these patients.

The decision of when to stop eculizumab is more difficult to determine in terms of the evidence available. Ultimately, the aim is to balance the risk of eculizumab (see side-effects) against effective treatment and prevention of recurrence of TMA and the organ-specific damage associated with it. In individuals where TMA has been controlled but renal function has not recovered eculizumab has usually been discontinued. There are, however, rare reports of severe extra-renal manifestations

such as cerebral artery stenoses which have been attributed to ongoing complement activation (101). The experience is currently too limited to recommend routine ongoing treatment with eculizumab in individuals on long term dialysis.

Ardinisso et al describe a small group of patients that discontinued eculizumab despite initial concerns that they would be at high risk of recurrence (97). This seems to have been well-tolerated with only 3 patients developing recurrent disease. However, knowing precisely which sub-group of patients would tolerate cessation, and at what time point into their treatment is hard to be certain. Further study is required.

Where genetic analysis reveals *DGKε* mutation, it is unlikely that complement inhibition will have affected the natural history of disease. In fact, there is at least one report of a patient that had recurrent disease whilst receiving eculizumab. Eculizumab should be stopped in these patients (99).

Pregnancy and Eculizumab in PNH and aHUS

Pregnancy is associated with poor outcomes in both PNH (102, 103) and aHUS (104). It is rarely reported with ~100 cases and ~20 respectively in the reported literature. Prior to the availability of eculizumab, PNH in pregnancy was more difficult to manage than usual due to increased haemolysis, transfusion requirements, tendency to thrombosis and ultimately increased maternal and foetal mortality (103). In aHUS triggered by pregnancy, the usual poor prognosis associated with aHUS is not diminished (104).

The introduction of eculizumab may be of particular benefit to this cohort of patients. The use of eculizumab in pregnancy in either disease is limited to small numbers. Hillmen et al report 7 patients who received eculizumab during pregnancy

(102). Of these, 3 remained on eculizumab at the time of delivery with successful outcomes. In aHUS, most patients develop aHUS in the post-partum period (104) reducing the concerns regarding safety during pregnancy. In the one case report that describes eculizumab during pregnancy and aHUS, haemolysis was controlled and there was improvement in renal function(105). There was no apparent complication.

Safety of using Eculizumab

The most extensive use of eculizumab is in patients with PNH. Hillmen et al (2013) reported the safety profile in their 195 patients that had received eculizumab during the open-label extension study (56). Eculizumab was well tolerated during the study. The most frequent adverse events (AE) were headache, nasopharyngitis and upper respiratory tract infection, reported in greater than 40% of patients. The majority of AE (91.3%) were mild to moderate in severity. Most of the serious AE were symptoms that are seen in PNH. These were haemolysis, abdominal pain and anaemia and were seen in 38.5% of patients. Only 5 patients discontinued eculizumab due to non-fatal AE. There was no comparator placebo arm in the extension study, but the investigators did compare the number of AE in the last 26 weeks of the study to the first 26 and found that the number of patients with 1 or more AE was significantly lower in the last 26 weeks suggesting there was no cumulative toxicity. Two patients had meningococcal infection and recovered following anti-microbial treatment. Though both had been vaccinated, neither had received vaccine for the strain that they actually developed.

Eculizumab was similarly well tolerated in the aHUS studies. Headache and upper respiratory tract infections were the most commonly reported in the adult phase 2 study (93). Additionally, 27 patients out of 37 had a serious AE, but all of

those that were possibly or probably associated with eculizumab resolved without cessation of treatment. There were no new serious AE after the initial 26 weeks of treatment. No patients developed meningococcal infection. All had received prophylactic anti-microbial treatment in addition to vaccination.

The use of eculizumab in the paediatric setting is more extensive in aHUS patients compared to PNH. In the paediatric study of aHUS patients, AE were similarly tolerated as reported in the adult studies. There were 2 patient deaths though they were not related to eculizumab (106). In the paediatric patients with PNH, there were no serious adverse events attributed to eculizumab.

Deposition of anti-C5 antibody on the glomerulus

In 3 patients who received eculizumab for complement-mediated glomerular disease, there was de-novo deposition of IgGκ in the glomerular tissue demonstrated on renal biopsy (107). Staining of the γ-heavy chain showed IgG2 and IgG4 subclasses consistent with deposition of eculizumab. The longer term effects of this interaction are not known.

Polymorphisms in C5 and resistance to Eculizumab

Certain individuals (11 out of 345 patients treated with eculizumab) in a Japanese PNH population did not respond to eculizumab despite adequate serum levels (108). In vitro assays of serum from these non-responders demonstrated an inability of eculizumab to prevent haemolysis. When an antibody against a different epitope on C5 was used, haemolysis was blocked. Genetic analysis revealed a SNP in C5 (p.R885H) in all non-responders (108). This SNP was present in around 3.2% of patients in this cohort, similar to the prevalence of 3.5% in the general Japanese

population. A further patient from Argentina (but also of Asian descent) presented similarly and had a mutation affecting the same amino acid (p.R885C) (108). Non-responders to eculizumab should have genetic screening for this SNP and alternative treatment strategies considered.

Cost of Eculizumab

Eculizumab is one of the more expensive drugs in use currently (109). The cost of eculizumab may alone prohibit its use in routine clinical practice. At the time of writing, National Institute for Clinical Excellence in the United Kingdom accepts the effectiveness of the drug (106) but cannot justify its use even in the small number of affected patients due to its expense.

Summary

Eculizumab is a first-in-class complement inhibitor developed to target the cleavage of C5 and therefore preventing the release of C5a and formation of MAC. It has been shown to be effective and safe in the treatment of both PNH and aHUS. Eculizumab exemplifies the clear benefits of targeted inhibition of the complement system. It is now being used in clinical services for both diseases and guidelines for use continue to evolve especially for aHUS in determining the likely duration of treatment.

However, eculizumab only inhibits the downstream effects of complement activation. Upstream of the site of action of eculizumab, there is ongoing complement activation. This results in the opsonisation of PNH erythrocytes leading

to uptake of these cells by the reticular endothelial system and extravascular haemolysis. In aHUS, eculizumab is not effective in patients who have mutations in genes in non-complement pathways such as *DGKε*. In PNH and aHUS, eculizumab is not effective in patients who carry a polymorphism in C5 affecting the epitope for eculizumab binding. Further studies will be required to determine whether eculizumab remains the optimal treatment for these two diseases.

Acknowledgements

EKSW is a Medical Research Council Clinical Research Training Fellow

DK is a Wellcome Trust Intermediate Clinical Fellow

Table 1

Complement regulator	**Function**	**Clinical association**		**Effect of mutation**
		Disease	**Mutation type**	
Factor H, FH	Decay of C3 convertase and co-factor activity for FI	aHUS (72)	FH, Germline	Loss of FH expression or function of circulating protein
		MPGN (110)		
		AMD (111)		
Factor I, FI	Proteolytic cleavage of C3b to iC3b and C3dg in the presence of co-factors	aHUS (74)	FI, Germline	Loss of FI expression or function of circulating protein
		AMD (112)		
Membrane co-factor, MCP, CD46	Co-factor activity for FI and C4-binding protein	aHUS (75)	CD46, Germline	Loss of CD46 expression or function of cell surface
Decay accelerating factor, DAF, CD55	Decay of C3 convertase	PNH (23)	*PIGA,* Somatic in haemopoietic stem cells	GPI-AP deficiency on surface of haemopoietic stem cell progeny
		Inab phenotype (113)	CD55,Germline	Loss of expression of CD55 on cell surface
CD59	Inhibition of MAC formation	PNH (23)	*PIGA,* Somatic in haemopoietic stem cells	GPI-AP deficiency on surface of haemopoietic stem cell progeny
		CD59 deficiency (114)	CD59, Germline	Loss of expression of CD59 on cell surface

Table 2

Study	Type of study	Number of patients	Duration of study in weeks (Range)	Effect of Eculizumab on			Thrombotic events during study	Other improvements in outcome
				Transfusion requirements	LDH	Proportion of Type 3 PNH erythrocytes		
Phase 2 pilot (47) and extension (16)	Single-arm open label and extension study	11	64	↓	↓	↑	0	QOL, haemoglobinuria
TRIUMPH Phase 3 (48)	Double blind RCT with placebo	87 (43 on Eculizumab)	26	↓	↓	↑	0	QOL
SHEPHERD (49)	Single arm open label	97	52	↓	↓	↑	2	QOL
UK (50)	Retrospective Study	79	39 (1-98)	↓	↓	N/A	1	Thrombotic events
Japan (52) and extension (53)	Single-arm open label and extension study	29	156	↓	↓	↑	0	QOL and CKD
US (54)	Retrospective study	30	24 (6-80)	↓	↓	↑	1	
Phase 1/2 Paediatric[a] (55)	Single-arm open label	7	12	Remained independent	↓	Stable	0	QOL

Table 3

Complement abnormality	Frequency of aHUS patients	Effect of mutation on function
FH	25%	Majority are C-terminal mutations leading to loss of binding to GAGs on glomerular endothelial surface; Others are N-terminal mutations leading to loss of fluid-phase complement regulation
FI	5-10%	Some cause loss of co-factor activity
CD46	10%	Affects binding of the extracellular domains to C3b resulting in loss of co-factor activity
C3	2-10%	C3 convertase that is more readily formed or are more resistant to decay acceleration
FB	Rare	C3 convertase that is more readily formed or are more resistant to decay acceleration
Auto-antibody to FH	4-14% (adult), 25% (paediatric)	Binds to epitopes on C-terminal of FH leading to loss of complement regulation. Other binding sites have been identified though function less certain

Table 4

Study	Type of Study	Number of patients	Duration	aHUS in native kidney (number of patients)	aHUS in transplanted kidney (number of patients)	Previous plasma exchange (number of patients)	Patients in whom platelet count normalised (%)	Patients achieving TMA free status[a] (%)	Improvement of CKD stage or eGFR>15ml/min (percentage of patients)
C08-002 (93)	Prospective	17	26 weeks	10	7	17	82	88	59[b]
C08-003 (93)	Prospective	20	26 weeks	12	8	20	90	80[c]	35[d]
C09-001 (95)	Retrospective	15	N/A	14[e]	1	N/A	93	80%	53
Fakhouri et al (94)	Retrospective	19	4-22months	19	0	16	100	100%	75[f]

References

1. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. Nat Immunol. 2010 Sep;11(9):785-97.
2. Kemper C, Atkinson JP. T-cell regulation: with complements from innate immunity. Nat Rev Immunol. 2007 Jan;7(1):9-18.
3. Richards A, Kavanagh D, Atkinson JP. Inherited complement regulatory protein deficiency predisposes to human disease in acute injury and chronic inflammatory statesthe examples of vascular damage in atypical hemolytic uremic syndrome and debris accumulation in age-related macular degeneration. Adv Immunol. 2007;96:141-77.
4. Harboe M, Mollnes TE. The alternative complement pathway revisited. J Cell Mol Med. 2008 Aug;12(4):1074-84.
5. Brodsky RA. Advances in the diagnosis and therapy of paroxysmal nocturnal hemoglobinuria. Blood Rev. 2008 Mar;22(2):65-74.
6. Kavanagh D, Goodship TH, Richards A. Atypical hemolytic uremic syndrome. Semin Nephrol. 2013 Nov;33(6):508-30.
7. Rother RP, Rollins SA, Mojcik CF, Brodsky RA, Bell L. Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria. Nat Biotechnol. 2007 Nov;25(11):1256-64.
8. Thomas TC, Rollins SA, Rother RP, Giannoni MA, Hartman SL, Elliott EA, et al. Inhibition of complement activity by humanized anti-C5 antibody and single-chain Fv. Mol Immunol. 1996 Dec;33(17-18):1389-401.
9. Canfield SM, Morrison SL. The binding affinity of human IgG for its high affinity Fc receptor is determined by multiple amino acids in the CH2 domain and is modulated by the hinge region. J Exp Med. 1991 Jun 1;173(6):1483-91.
10. Tao MH, Smith RI, Morrison SL. Structural features of human immunoglobulin G that determine isotype-specific differences in complement activation. J Exp Med. 1993 Aug 1;178(2):661-7.
11. Wang Y, Rollins SA, Madri JA, Matis LA. Anti-C5 monoclonal antibody therapy prevents collagen-induced arthritis and ameliorates established disease. Proc Natl Acad Sci U S A. 1995 Sep 12;92(19):8955-9.
12. Wang Y, Hu Q, Madri JA, Rollins SA, Chodera A, Matis LA. Amelioration of lupus-like autoimmune disease in NZB/WF1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. Proc Natl Acad Sci U S A. 1996 Aug 6;93(16):8563-8.
13. Rother RP, Mojcik CF, McCroskery EW. Inhibition of terminal complement: a novel therapeutic approach for the treatment of systemic lupus erythematosus. Lupus. 2004;13(5):328-34.
14. Jain RI, Moreland LW, Caldwell JR, Rollins SA, Mojcik CF. A single dose, placebo controlled, double blind, phase I study of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis. Arthritis and Rheumatism. 1999 Sep;42(9):S77-S.
15. Tesser J, Kivitz A, Fleischmann R, Mojcik CF, Bombara M, Burch F. Safety and efficacy of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis. Arthritis and Rheumatism. 2001 Sep;44(9):S274-S.
16. Hill A, Hillmen P, Richards SJ, Elebute D, Marsh JC, Chan J, et al. Sustained response and long-term safety of eculizumab in paroxysmal nocturnal hemoglobinuria. Blood. 2005 Oct 1;106(7):2559-65.
17. Figueroa JE, Densen P. Infectious diseases associated with complement deficiencies. Clin Microbiol Rev. 1991 Jul;4(3):359-95.
18. Strubing P. Paroxysmale Haemoglobinurie. Deutsche Medicinische Wochenschrift. 1882;8:1-16.
19. Dacie JV, Lewis SM. Paroxysmal nocturnal haemoglobinuria: clinical manifestations, haematology, and nature of the disease. Ser Haematol. 1972;5(3):3-23.

20. Parker C, Omine M, Richards S, Nishimura J, Bessler M, Ware R, et al. Diagnosis and management of paroxysmal nocturnal hemoglobinuria. Blood. 2005 Dec 1;106(12):3699-709.
21. Ham TH, Dingle JH. Studies on Destruction of Red Blood Cells. Ii. Chronic Hemolytic Anemia with Paroxysmal Nocturnal Hemoglobinuria: Certain Immunological Aspects of the Hemolytic Mechanism with Special Reference to Serum Complement. J Clin Invest. 1939 Nov;18(6):657-72.
22. Pillemer L, Blum L, Lepow IH, Ross OA, Todd EW, Wardlaw AC. The properdin system and immunity. I. Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. Science. 1954 Aug 20;120(3112):279-85.
23. Parker CJ. The pathophysiology of paroxysmal nocturnal hemoglobinuria. Exp Hematol. 2007 Apr;35(4):523-33.
24. Kinoshita T, Inoue N, Takeda J. Defective glycosyl phosphatidylinositol anchor synthesis and paroxysmal nocturnal hemoglobinuria. Adv Immunol. 1995;60:57-103.
25. Araten DJ, Nafa K, Pakdeesuwan K, Luzzatto L. Clonal populations of hematopoietic cells with paroxysmal nocturnal hemoglobinuria genotype and phenotype are present in normal individuals. Proc Natl Acad Sci U S A. 1999 Apr 27;96(9):5209-14.
26. Mukhina GL, Buckley JT, Barber JP, Jones RJ, Brodsky RA. Multilineage glycosylphosphatidylinositol anchor-deficient haematopoiesis in untreated aplastic anaemia. Br J Haematol. 2001 Nov;115(2):476-82.
27. Inoue N, Izui-Sarumaru T, Murakami Y, Endo Y, Nishimura J, Kurokawa K, et al. Molecular basis of clonal expansion of hematopoiesis in 2 patients with paroxysmal nocturnal hemoglobinuria (PNH). Blood. 2006 Dec 15;108(13):4232-6.
28. Brodsky RA, Vala MS, Barber JP, Medof ME, Jones RJ. Resistance to apoptosis caused by PIG-A gene mutations in paroxysmal nocturnal hemoglobinuria. Proc Natl Acad Sci U S A. 1997 Aug 5;94(16):8756-60.
29. Young NS. Paroxysmal nocturnal hemoglobinuria and myelodysplastic syndromes: clonal expansion of PIG-A-mutant hematopoietic cells in bone marrow failure. Haematologica. 2009 Jan;94(1):3-7.
30. Nowak J, Wozniak J, Mendek-Czajkowska E, Dlugokecka A, Mika-Witkowska R, Rogatko-Koros M, et al. Potential link between MHC-self-peptide presentation and hematopoiesis; the analysis of HLA-DR expression in CD34-positive cells and self-peptide presentation repertoires of MHC molecules associated with paroxysmal nocturnal hemoglobinuria. Cell Biochem Biophys. 2013 Apr;65(3):321-33.
31. Rosse WF. Paroxysmal nocturnal hemoglobinuria--present status and future prospects. West J Med. 1980 Mar;132(3):219-28.
32. Hill A, Kelly RJ, Hillmen P. Thrombosis in paroxysmal nocturnal hemoglobinuria. Blood. 2013 Jun 20;121(25):4985-96; quiz 5105.
33. Devine DV, Siegel RS, Rosse WF. Interactions of the platelets in paroxysmal nocturnal hemoglobinuria with complement. Relationship to defects in the regulation of complement and to platelet survival in vivo. J Clin Invest. 1987 Jan;79(1):131-7.
34. Wiedmer T, Hall SE, Ortel TL, Kane WH, Rosse WF, Sims PJ. Complement-induced vesiculation and exposure of membrane prothrombinase sites in platelets of paroxysmal nocturnal hemoglobinuria. Blood. 1993 Aug 15;82(4):1192-6.
35. Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. JAMA. 2005 Apr 6;293(13):1653-62.
36. Gralnick HR, Vail M, McKeown LP, Merryman P, Wilson O, Chu I, et al. Activated platelets in paroxysmal nocturnal haemoglobinuria. Br J Haematol. 1995 Nov;91(3):697-702.
37. Ploug M, Plesner T, Ronne E, Ellis V, Hoyer-Hansen G, Hansen NE, et al. The receptor for urokinase-type plasminogen activator is deficient on peripheral blood leukocytes in patients with paroxysmal nocturnal hemoglobinuria. Blood. 1992 Mar 15;79(6):1447-55.

38. Maroney SA, Cunningham AC, Ferrel J, Hu R, Haberichter S, Mansbach CM, et al. A GPI-anchored co-receptor for tissue factor pathway inhibitor controls its intracellular trafficking and cell surface expression. J Thromb Haemost. 2006 May;4(5):1114-24.
39. Clark DA, Butler SA, Braren V, Hartmann RC, Jenkins DE, Jr. The kidneys in paroxysmal nocturnal hemoglobinuria. Blood. 1981 Jan;57(1):83-9.
40. Nath KA, Vercellotti GM, Grande JP, Miyoshi H, Paya CV, Manivel JC, et al. Heme protein-induced chronic renal inflammation: Suppressive effect of induced heme oxygenase-1. Kidney International. 2001 Jan;59(1):106-17.
41. Schlaich MP, Schmitt D, Ott C, Schmidt BM, Schmieder RE. Basal nitric oxide synthase activity is a major determinant of glomerular haemodynamics in humans. J Hypertens. 2008 Jan;26(1):110-6.
42. Richards SJ, Rawstron AC, Hillmen P. Application of flow cytometry to the diagnosis of paroxysmal nocturnal hemoglobinuria. Cytometry. 2000 Aug 15;42(4):223-33.
43. Hillmen P, Lewis SM, Bessler M, Luzzatto L, Dacie JV. Natural history of paroxysmal nocturnal hemoglobinuria. N Engl J Med. 1995 Nov 9;333(19):1253-8.
44. Araten DJ, Thaler HT, Luzzatto L. High incidence of thrombosis in African-American and Latin-American patients with Paroxysmal Nocturnal Haemoglobinuria. Thromb Haemost. 2005 Jan;93(1):88-91.
45. Nishimura J, Kanakura Y, Ware RE, Shichishima T, Nakakuma H, Ninomiya H, et al. Clinical course and flow cytometric analysis of paroxysmal nocturnal hemoglobinuria in the United States and Japan. Medicine (Baltimore). 2004 May;83(3):193-207.
46. Hillmen P, Elebute M, Kelly R, Urbano-Ispizua A, Hill A, Rother RP, et al. Long-term effect of the complement inhibitor eculizumab on kidney function in patients with paroxysmal nocturnal hemoglobinuria. Am J Hematol. 2010 Aug;85(8):553-9.
47. Hillmen P, Hall C, Marsh JC, Elebute M, Bombara MP, Petro BE, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. N Engl J Med. 2004 Feb 5;350(6):552-9.
48. Hillmen P, Young NS, Schubert J, Brodsky RA, Socie G, Muus P, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. N Engl J Med. 2006 Sep 21;355(12):1233-43.
49. Brodsky RA, Young NS, Antonioli E, Risitano AM, Schrezenmeier H, Schubert J, et al. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. Blood. 2008 Feb 15;111(4):1840-7.
50. Kelly RJ, Hill A, Arnold LM, Brooksbank GL, Richards SJ, Cullen M, et al. Long-term treatment with eculizumab in paroxysmal nocturnal hemoglobinuria: sustained efficacy and improved survival. Blood. 2011 Jun 23;117(25):6786-92.
51. Lopez Rubio M, Morado M, Gaya A, Alonso Rosa D, Ojeda E, Munoz JA, et al. [Paroxysmal nocturnal hemoglobinuria therapy with eculizumab: Spanish experience]. Med Clin (Barc). 2011 Jun 11;137(1):8-13.
52. Kanakura Y, Ohyashiki K, Shichishima T, Okamoto S, Ando K, Ninomiya H, et al. Safety and efficacy of the terminal complement inhibitor eculizumab in Japanese patients with paroxysmal nocturnal hemoglobinuria: the AEGIS clinical trial. Int J Hematol. 2011 Jan;93(1):36-46.
53. Kanakura Y, Ohyashiki K, Shichishima T, Okamoto S, Ando K, Ninomiya H, et al. Long-term efficacy and safety of eculizumab in Japanese patients with PNH: AEGIS trial. Int J Hematol. 2013 Oct;98(4):406-16.
54. DeZern AE, Dorr D, Brodsky RA. Predictors of hemoglobin response to eculizumab therapy in paroxysmal nocturnal hemoglobinuria. Eur J Haematol. 2013 Jan;90(1):16-24.
55. Reiss UM, Schwartz J, Sakamoto KM, Puthenveetil G, Ogawa M, Bedrosian CL, et al. Efficacy and safety of eculizumab in children and adolescents with paroxysmal nocturnal hemoglobinuria. Pediatr Blood Cancer. 2014 Apr 29.

56. Hillmen P, Muus P, Roth A, Elebute MO, Risitano AM, Schrezenmeier H, et al. Long-term safety and efficacy of sustained eculizumab treatment in patients with paroxysmal nocturnal haemoglobinuria. Br J Haematol. 2013 Jul;162(1):62-73.
57. Hill A, Rother RP, Wang X, Morris SM, Jr., Quinn-Senger K, Kelly R, et al. Effect of eculizumab on haemolysis-associated nitric oxide depletion, dyspnoea, and measures of pulmonary hypertension in patients with paroxysmal nocturnal haemoglobinuria. Br J Haematol. 2010 May;149(3):414-25.
58. Hill A, Rother RP, Hillmen P. Improvement in the symptoms of smooth muscle dystonia during eculizumab therapy in paroxysmal nocturnal hemoglobinuria. Haematologica. 2005 Dec;90(12 Suppl):ECR40.
59. Risitano AM, Notaro R, Marando L, Serio B, Ranaldi D, Seneca E, et al. Complement fraction 3 binding on erythrocytes as additional mechanism of disease in paroxysmal nocturnal hemoglobinuria patients treated by eculizumab. Blood. 2009 Apr 23;113(17):4094-100.
60. Hill A, Rother RP, Arnold L, Kelly R, Cullen MJ, Richards SJ, et al. Eculizumab prevents intravascular hemolysis in patients with paroxysmal nocturnal hemoglobinuria and unmasks low-level extravascular hemolysis occurring through C3 opsonization. Haematologica. 2010 Apr;95(4):567-73.
61. Rondelli T, Risitano AM, Peffault de Latour R, Sica M, Peruzzi B, Ricci P, et al. Polymorphism of the complement receptor 1 gene correlates with the hematologic response to eculizumab in patients with paroxysmal nocturnal hemoglobinuria. Haematologica. 2014 Feb;99(2):262-6.
62. Lindorfer MA, Pawluczkowycz AW, Peek EM, Hickman K, Taylor RP, Parker CJ. A novel approach to preventing the hemolysis of paroxysmal nocturnal hemoglobinuria: both complement-mediated cytolysis and C3 deposition are blocked by a monoclonal antibody specific for the alternative pathway of complement. Blood. 2010 Mar 18;115(11):2283-91.
63. Schmidt CQ, Bai H, Lin Z, Risitano AM, Barlow PN, Ricklin D, et al. Rational engineering of a minimized immune inhibitor with unique triple-targeting properties. J Immunol. 2013 Jun 1;190(11):5712-21.
64. Fridkis-Hareli M, Storek M, Mazsaroff I, Risitano AM, Lundberg AS, Horvath CJ, et al. Design and development of TT30, a novel C3d-targeted C3/C5 convertase inhibitor for treatment of human complement alternative pathway-mediated diseases. Blood. 2011 Oct 27;118(17):4705-13.
65. Risitano AM, Ricklin D, Huang Y, Reis ES, Chen H, Ricci P, et al. Peptide inhibitors of C3 activation as a novel strategy of complement inhibition for the treatment of paroxysmal nocturnal hemoglobinuria. Blood. 2014 Feb 4.
66. Lee M, Narayanan S, McGeer EG, McGeer PL. Aurin tricarboxylic acid protects against red blood cell hemolysis in patients with paroxysmal nocturnal hemoglobinemia. PLoS One. 2014;9(1):e87316.
67. England N. 2013/14 NHS Strandard Contract for Paroxysmal Noctural Haemoglobinuria Serives (Adults and Adolescents). 2013 [cited 2014 May 27th]; Available from: http://www.england.nhs.uk/wp-content/uploads/2013/06/b05-parox-haem-serv.pdf.
68. Kavanagh D, Richards A, Atkinson J. Complement regulatory genes and hemolytic uremic syndromes. Annu Rev Med. 2008;59:293-309.
69. Besbas N, Karpman D, Landau D, Loirat C, Proesmans W, Remuzzi G, et al. A classification of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura and related disorders. Kidney Int. 2006 Aug;70(3):423-31.
70. Loirat C, Fremeaux-Bacchi V. Atypical hemolytic uremic syndrome. Orphanet J Rare Dis. 2011;6:60.
71. Schmidt CQ, Herbert AP, Hocking HG, Uhrin D, Barlow PN. Translational mini-review series on complement factor H: structural and functional correlations for factor H. Clin Exp Immunol. [Review]. 2008 Jan;151(1):14-24.

72. Ferreira VP, Herbert AP, Cortes C, McKee KA, Blaum BS, Esswein ST, et al. The binding of factor H to a complex of physiological polyanions and C3b on cells is impaired in atypical hemolytic uremic syndrome. J Immunol. 2009 Jun 1;182(11):7009-18.
73. Warwicker P, Goodship TH, Donne RL, Pirson Y, Nicholls A, Ward RM, et al. Genetic studies into inherited and sporadic hemolytic uremic syndrome. Kidney Int. 1998 Apr;53(4):836-44.
74. Kavanagh D, Kemp EJ, Mayland E, Winney RJ, Duffield JS, Warwick G, et al. Mutations in complement factor I predispose to development of atypical hemolytic uremic syndrome. J Am Soc Nephrol. 2005 Jul;16(7):2150-5.
75. Richards A, Kathryn Liszewski M, Kavanagh D, Fang CJ, Moulton E, Fremeaux-Bacchi V, et al. Implications of the initial mutations in membrane cofactor protein (MCP; CD46) leading to atypical hemolytic uremic syndrome. Mol Immunol. 2007 Jan;44(1-3):111-22.
76. Frémeaux-Bacchi V, Miller EC, Liszewski MK, Strain L, Blouin J, Brown AL, et al. Mutations in complement C3 predispose to development of atypical hemolytic uremic syndrome. Blood. 2008 December 15, 2008;112(13):4948-52.
77. Goicoechea de Jorge E, Harris CL, Esparza-Gordillo J, Carreras L, Arranz EA, Garrido CA, et al. Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome. Proc Natl Acad Sci U S A. 2007 Jan 2;104(1):240-5.
78. Jozsi M, Strobel S, Dahse HM, Liu WS, Hoyer PF, Oppermann M, et al. Anti factor H autoantibodies block C-terminal recognition function of factor H in hemolytic uremic syndrome. Blood. 2007 Sep 1;110(5):1516-8.
79. Blanc C, Roumenina LT, Ashraf Y, Hyvarinen S, Sethi SK, Ranchin B, et al. Overall neutralization of complement factor H by autoantibodies in the acute phase of the autoimmune form of atypical hemolytic uremic syndrome. J Immunol. 2012 Oct 1;189(7):3528-37.
80. Kavanagh D, Pappworth IY, Anderson H, Hayes CM, Moore I, Hunze EM, et al. Factor I autoantibodies in patients with atypical hemolytic uremic syndrome: disease-associated or an epiphenomenon? Clin J Am Soc Nephrol. 2012 Mar;7(3):417-26.
81. Pickering MC, de Jorge EG, Martinez-Barricarte R, Recalde S, Garcia-Layana A, Rose KL, et al. Spontaneous hemolytic uremic syndrome triggered by complement factor H lacking surface recognition domains. J Exp Med. 2007 Jun 11;204(6):1249-56.
82. Fremeaux-Bacchi V, Kemp EJ, Goodship JA, Dragon-Durey MA, Strain L, Loirat C, et al. The development of atypical haemolytic-uraemic syndrome is influenced by susceptibility factors in factor H and membrane cofactor protein: evidence from two independent cohorts. J Med Genet. 2005 Nov;42(11):852-6.
83. Caprioli J, Noris M, Brioschi S, Pianetti G, Castelletti F, Bettinaglio P, et al. Genetics of HUS: the impact of MCP, CFH, and IF mutations on clinical presentation, response to treatment, and outcome. Blood. 2006 Aug 15;108(4):1267-79.
84. Bresin E, Rurali E, Caprioli J, Sanchez-Corral P, Fremeaux-Bacchi V, Rodriguez de Cordoba S, et al. Combined complement gene mutations in atypical hemolytic uremic syndrome influence clinical phenotype. J Am Soc Nephrol. 2013 Feb;24(3):475-86.
85. Sellier-Leclerc AL, Fremeaux-Bacchi V, Dragon-Durey MA, Macher MA, Niaudet P, Guest G, et al. Differential impact of complement mutations on clinical characteristics in atypical hemolytic uremic syndrome. J Am Soc Nephrol. 2007 Aug;18(8):2392-400.
86. Hosler GA, Cusumano AM, Hutchins GM. Thrombotic thrombocytopenic purpura and hemolytic uremic syndrome are distinct pathologic entities. A review of 56 autopsy cases. Arch Pathol Lab Med. 2003 Jul;127(7):834-9.
87. Fremeaux-Bacchi V, Fakhouri F, Garnier A, Bienaime F, Dragon-Durey MA, Ngo S, et al. Genetics and outcome of atypical hemolytic uremic syndrome: a nationwide French series comparing children and adults. Clin J Am Soc Nephrol. 2013 Apr;8(4):554-62.
88. Le Quintrec M, Zuber J, Moulin B, Kamar N, Jablonski M, Lionet A, et al. Complement genes strongly predict recurrence and graft outcome in adult renal transplant recipients with atypical hemolytic and uremic syndrome. Am J Transplant. 2013 Mar;13(3):663-75.

89. de Jorge EG, Macor P, Paixao-Cavalcante D, Rose KL, Tedesco F, Cook HT, et al. The development of atypical hemolytic uremic syndrome depends on complement C5. J Am Soc Nephrol. 2011 Jan;22(1):137-45.
90. Gruppo RA, Rother RP. Eculizumab for congenital atypical hemolytic-uremic syndrome. N Engl J Med. 2009 Jan 29;360(5):544-6.
91. Nurnberger J, Philipp T, Witzke O, Opazo Saez A, Vester U, Baba HA, et al. Eculizumab for atypical hemolytic-uremic syndrome. N Engl J Med. 2009 Jan 29;360(5):542-4.
92. Wong EK, Goodship TH, Kavanagh D. Complement therapy in atypical haemolytic uraemic syndrome (aHUS). Mol Immunol. 2013 Dec 15;56(3):199-212.
93. Legendre CM, Licht C, Muus P, Greenbaum LA, Babu S, Bedrosian C, et al. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. N Engl J Med. 2013 Jun 6;368(23):2169-81.
94. Fakhouri F, Delmas Y, Provot F, Barbet C, Karras A, Makdassi R, et al. Insights from the use in clinical practice of eculizumab in adult patients with atypical hemolytic uremic syndrome affecting the native kidneys: an analysis of 19 cases. Am J Kidney Dis. 2014 Jan;63(1):40-8.
95. Alexion. SPC Soliris. 2014 [cited 2014 May 27th]; Available from: http://www.medicines.org.uk/emc/medicine/19966.
96. Povey H, Vundru R, Junglee N, Jibani M. Renal recovery with eculizumab in atypical hemolytic uremic syndrome following prolonged dialysis. Clin Nephrol. 2013 Apr 5.
97. Ardissino G, Testa S, Possenti I, Tel F, Paglialonga F, Salardi S, et al. Discontinuation of Eculizumab Maintenance Treatment for Atypical Hemolytic Uremic Syndrome: A Report of 10 Cases. Am J Kidney Dis. 2014 Mar 19.
98. Ozaltin F, Li B, Rauhauser A, An SW, Soylemezoglu O, Gonul, II, et al. DGKE variants cause a glomerular microangiopathy that mimics membranoproliferative GN. J Am Soc Nephrol. 2013 Feb;24(3):377-84.
99. Lemaire M, Fremeaux-Bacchi V, Schaefer F, Choi M, Tang WH, Le Quintrec M, et al. Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. Nat Genet. 2013 May;45(5):531-6.
100. George JN, Nester CM. Syndromes of thrombotic microangiopathy. N Engl J Med. 2014 Aug 14;371(7):654-66.
101. Loirat C, Macher MA, Elmaleh-Berges M, Kwon T, Deschenes G, Goodship TH, et al. Non-atheromatous arterial stenoses in atypical haemolytic uraemic syndrome associated with complement dysregulation. Nephrol Dial Transplant. 2010 Oct;25(10):3421-5.
102. Kelly R, Arnold L, Richards S, Hill A, Bomken C, Hanley J, et al. The management of pregnancy in paroxysmal nocturnal haemoglobinuria on long term eculizumab. Br J Haematol. 2010 May;149(3):446-50.
103. Danilov AV, Brodsky RA, Craigo S, Smith H, Miller KB. Managing a pregnant patient with paroxysmal nocturnal hemoglobinuria in the era of eculizumab. Leuk Res. 2010 May;34(5):566-71.
104. Fakhouri F, Roumenina L, Provot F, Sallee M, Caillard S, Couzi L, et al. Pregnancy-associated hemolytic uremic syndrome revisited in the era of complement gene mutations. J Am Soc Nephrol. 2010 May;21(5):859-67.
105. Ardissino G, Wally Ossola M, Baffero GM, Rigotti A, Cugno M. Eculizumab for atypical hemolytic uremic syndrome in pregnancy. Obstet Gynecol. 2013 Aug;122(2 Pt 2):487-9.
106. Rathbone J, Kaltenthaler E, Richards A, Tappenden P, Bessey A, Cantrell A. A systematic review of eculizumab for atypical haemolytic uraemic syndrome (aHUS). BMJ Open. 2013;3(11):e003573.
107. Herlitz LC, Bomback AS, Markowitz GS, Stokes MB, Smith RN, Colvin RB, et al. Pathology after eculizumab in dense deposit disease and C3 GN. J Am Soc Nephrol. 2012 Jul;23(7):1229-37.
108. Nishimura J, Yamamoto M, Hayashi S, Ohyashiki K, Ando K, Brodsky AL, et al. Genetic variants in C5 and poor response to eculizumab. N Engl J Med. 2014 Feb 13;370(7):632-9.
109. British national formulary: London : British Medical Association : Pharmaceutical Society of Great Britain.

110. Wong EK, Anderson HE, Herbert AP, Challis RC, Brown P, Reis GS, et al. Characterization of a Factor H Mutation That Perturbs the Alternative Pathway of Complement in a Family with Membranoproliferative GN. J Am Soc Nephrol. 2014 Apr 10.
111. Yu Y, Triebwasser MP, Wong EK, Schramm EC, Thomas B, Reynolds R, et al. Whole -exome sequencing identifies rare, functional CFH variants in families with macular degeneration. Hum Mol Genet. 2014 May 20.
112. Seddon JM, Yu Y, Miller EC, Reynolds R, Tan PL, Gowrisankar S, et al. Rare variants in CFI, C3 and C9 are associated with high risk of advanced age-related macular degeneration. Nat Genet. 2013 Nov;45(11):1366-70.
113. Telen MJ, Green AM. The Inab phenotype: characterization of the membrane protein and complement regulatory defect. Blood. 1989 Jul;74(1):437-41.
114. Nevo Y, Ben-Zeev B, Tabib A, Straussberg R, Anikster Y, Shorer Z, et al. CD59 deficiency is associated with chronic hemolysis and childhood relapsing immune-mediated polyneuropathy. Blood. 2013 Jan 3;121(1):129-35.

Figures and Tables

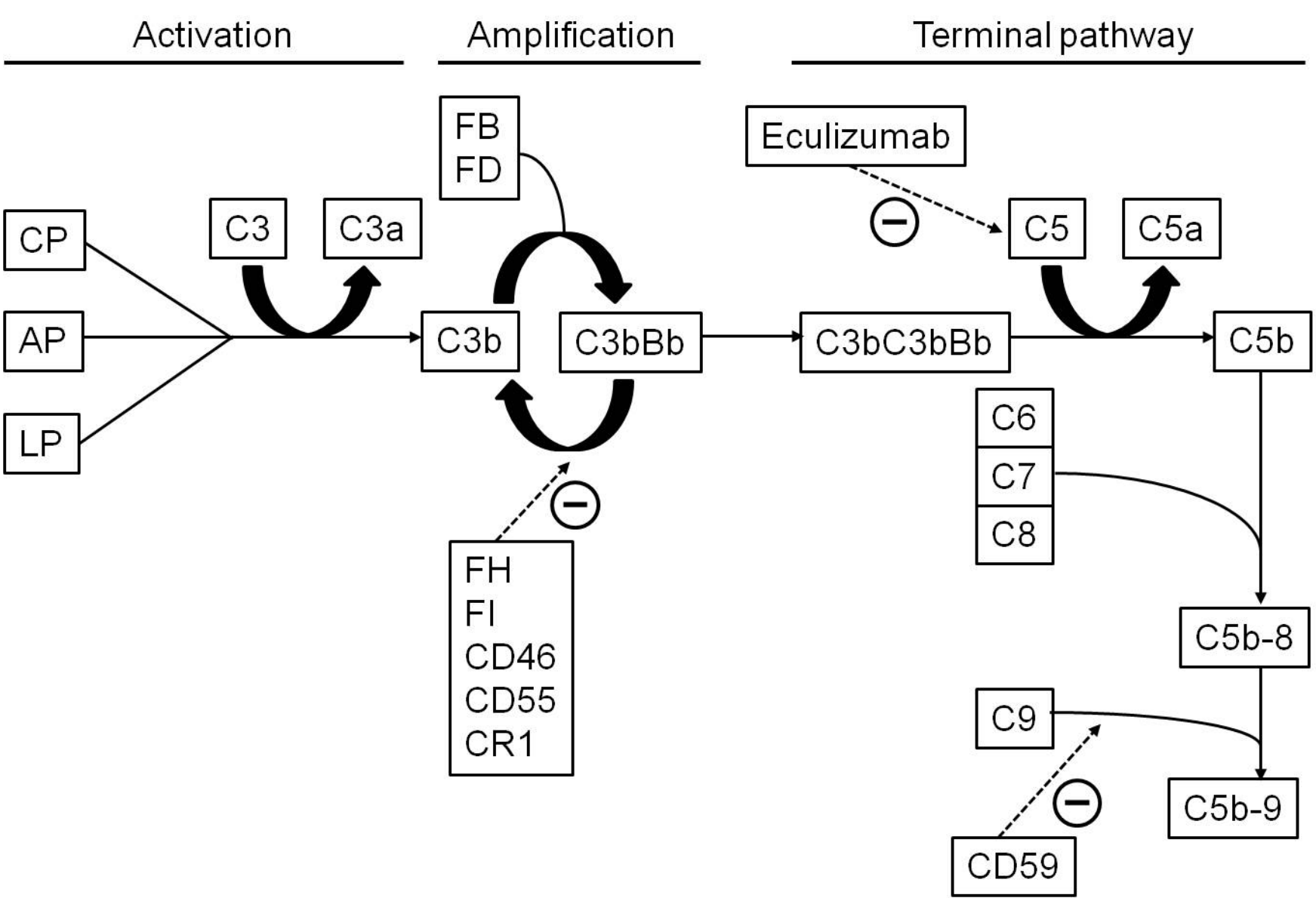
Figure 1. The complement system. Activation of complement C3 to form C3b can occur via the three pathways, classical (CP), alternative (AP) and lectin (AP). There is amplification of C3b by formation of the C3 convertase (C3bBb) in a positive feedback loop. C5 convertases (C3bC3bBb) then form and cleave C5 into C5a and C5b. C5b then enters the terminal pathway to bind C6, C7, C8 and finally C9 to form the membrane attack complex (MAC, C5b-9). The sites of action of complement regulators (FH, FI, CD46, CD55, CR1 and CD59) or inhibitor (Eculizumab) are indicated by the dotted arrow. The specific function of the complement regulators is described in tables 1 and 3. In PNH, CD59 deficiency on the surface of PNH erythrocytes leaves the erythrocyte vulnerable to MAC formation and lysis. In aHUS, mutations FH, FI and CD46 lead to loss of regulation of C3b activation and amplification. Conversely mutations in C3 and FB lead to hyperfunctioning complement components leading to over-activity of C3 convertase. Eculizumab is a monoclonal antibody that binds to C5 and blocks cleavage of C5 and therefore prevents MAC formation.

Table 1. Complement regulators, function and disease association. MAC - membrane attack complex; aHUS – atypical haemolytic uraemic syndrome; MPGN – membranoproliferative glomerulonephritis; AMD – age-related macular degeneration; PNH – paroxysmal nocturnal haemoglobinuria; PIG-A - *phosphatidylinositol glycan class A*; GPI-AP - glycosyl phosphatidylinositol anchored proteins

Table 2 Studies of Eculizumab for the treatment of haemolytic anaemia in paroxysmal nocturnal haemoglobinuria (PNH). In all studies, the dose of Eculizumab was 600mg weekly (x4) then 900mg (x1) then 900mg fortnightly. [a] All patients weighed >30kg and were dosed as per the standard regime. LDH – lactate dehydrogenase; QOL – quality of life; RCT – randomised controlled trial; N/A – not available; CKD – chronic kidney disease

Table 3. Complement abnormalities in aHUS, frequency and functional significance. FH – Factor H; FI – Factor I; FB – Factor B; GAG – glycosaminoglycans

Table 4 Studies of Eculizumab for the treatment of atypical haemolytic uraemic syndrome (aHUS) . In the adult studies, the dose of Eculizumab 900mg weekly (x4), 1200 mg (x1) then 1200mg fortnightly. Children under 40kg receive a weight-based dose (95). [a]defined as ≥12 weeks of stable platelet count, no plasma exchange or infusion and no new dialysis. [b] increased to 65% at 64 weeks. [c] increased to 85% at 62 weeks. [d] increased to 45% at 62 weeks. [e]assumed to be 14 patients. [f]percentage of patients that became independent of dialysis at 3 months. N/A – not available; TMA – thrombotic microangiopathy; CKD – chronic kidney disease; eGFR – estimated glomerular filtration rate


Activation
Amplification
Terminal pathway
CP
AP
LP
C3
C3a
FB
FD
C3b
C3bBb
FH
FI
CD46
CD55
CR1
Eculizumab
C5
C5a
C3bC3bBb
C5b
C6
C7
C8
C5b-8
C9
C5b-9
CD59